Detection of *Giardia duodenalis* assemblage A and B isolates by immunochromatography in stool samples from Rwandan children

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Abstract

We evaluated the performance of an immunochromatographic assay (ICA) in comparison with light microscopy and PCR for the detection of *Giardia duodenalis* in stool samples from 558 Rwandan children. The association of infection with clinical symptoms was similar for the three diagnostic tools. The ICA equally detected parasites of assemblages A and B and was more sensitive than light microscopy (50.4 versus 29.5% of PCR-positive samples considered true positive; $p < 0.0001$). Hence, the ICA shows superior sensitivity compared with microscopy but still misses half of the *G. duodenalis* infections detected by PCR in this hyperendemic area.

Keywords: Genotypes, *Giardia duodenalis*, immunochromatography, microscopy, PCR, Rwanda
Original Submission: 3 January 2014; **Revised Submission:** 11 February 2014; **Accepted:** 11 February 2014
Editor: E. Bottieau
Article published online: 17 February 2014
Clin Microbiol Infect 2014; **20**: O783–O784
10.1111/1469-0691.12596

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Giardia duodenalis is the most frequent parasite causing gastroenteritis worldwide, and asymptomatic infection is common in hyperendemic regions. Eight major genetic groups have been described (assemblages A–H; of which A and B are pathogenic in humans), which may represent different species [1]. Prevalence differs widely and by PCR, we and others have shown >60% of children in East Africa to be infected [2,3]. Most of these infections were not detected by light microscopy, which constitutes the only diagnostic method available in many resource-poor areas. Alternative methods, e.g. direct fluorescence, immunoassays and PCR [4–6], are difficult to establish and maintain in resource-poor settings and often require technologically advanced equipment. Antigen-detecting immunochromatographic assays (ICAs) may be an alternative because they (i) are easy and rapid to perform, (ii) can be applied on frozen samples and (iii) are independent of further equipment [7–10]. Most studies evaluating ICAs, however, were performed on autochthonous cases in developed countries or in travellers who might shed more parasites than chronically/repeatedly infected individuals in hyperendemic areas. Here, we compared an ICA with microscopy and PCR for the detection of *G. duodenalis* in stool samples from Rwandan children.

In 2010, 583 largely asymptomatic children under 5 years of age were recruited in the area of Butare, southern Rwanda. Study area, sampling, sample collection and examinations have been detailed previously [3]. The study was approved by the National Ethics Committee, Republic of Rwanda, and children's parents gave informed written consent. Children with microscopically detected *G. duodenalis* were treated with metronidazole.

Triplicate light microscopy of identical samples was performed in Rwanda and Germany. Stool samples were stored at –70°C. For ICA, thawed stool samples were immediately subjected to a commercially available assay (Rida Quick Giardia; R-Biopharm, Darmstadt, Germany; sensitivity, 100%, specificity, 95.2% compared with light microscopy; information provided by the manufacturer). DNA was extracted from thawed stool samples (Qiamp DNA Stool Mini Kit; Qiagen, Hilden, Germany), and a multiplex real-time PCR assay was performed to identify *G. duodenalis* [5]. For samples that were positive with ICA but PCR-negative, both assays were repeated. Assemblage typing was performed as described previously [3].

For the present analysis, stool samples of 558 children were available. We set *G. duodenalis* detected by PCR as reference and calculated sensitivity, specificity, positive predictive value and negative predictive value for microscopy and ICA, respectively. Values of $p < 0.05$ were considered statistically significant. We also compared the sensitivities regarding assemblages A and B, and the clinical characteristics of infected children.

Socio-demographic characteristics of the study population have been presented previously [3]. Briefly, the majority of children (median age, 32 months; range, 0.5–60 months; 46% female) were from poor, rural farming communities (84%). For the analysis of clinical characteristics, 474 community children

were considered because of the diverse additional morbidity of health-facility children [3].

Immunochromatography assay detected half of the *G. duodenalis* infections defined by positive PCR but microscopy detected less than a third. This difference in sensitivity was also reflected by PCR Ct values: for samples positive by PCR only, positive by PCR and ICA but negative by microscopy, and for microscopically positive samples, these were (mean ± SD) 33.2 ± 2.6, 29.1 ± 3.9, and 24.2 ± 32, respectively ($p < 0.0001$). All three methods identified *G. duodenalis* infection as being associated with severe malnutrition, although the strength of association tended to increase with declining sensitivity (Table 1). Abdominal distension was most strongly linked with *G. duodenalis* infection detected by PCR, followed by infection detected by ICA, and only tended to be linked in microscopically positive infections. No differences regarding fever, loss of appetite, diarrhoea, vomiting, abdominal pain, or being underweight were observed (Table 1). Compared with PCR, sensitivity ($p < 0.0001$) and negative predictive value (p 0.02) of the ICA were higher than those of microscopy (Table 2).

Assemblage typing was successful in 200 of 359 PCR-positive samples, of which 77.0% and 50.0% were positive by ICA and microscopy, respectively. Eighty-six per cent (172/200) of the isolates were of assemblage B. The sensitivity in detecting these was higher for the ICA (75.6%; 95% CI 68.3–81.7%) than for microscopy (45.9, 95% CI 38.4–53.7; $p < 0.0001$) whereas the sensitivities in detecting assemblage A parasites were similar (85.7%, 95% CI 66.4–95.3% versus 75.0%, 95% CI 68.3–81.7%). This is plausible because samples typed as assemblage A contained more parasites on average than B-typed samples [3].

In this study, the ICA was more sensitive than microscopy and detected assemblage A and B parasites equally. Microscopy failed to detect a large proportion of the predominating assemblage B isolates, most likely because of their comparatively lower density. The only other study on *G. duodenalis* ICA in a resource-poor area, in Bangladesh, reported considerable agreement between three rapid tests and ELISA [11]. However, as the authors only tested 18 discrepant samples with PCR, the sensitivities of ICA versus PCR could not reliably be estimated in that study.

A Spanish study reported strong agreement of results from PCR and microscopy with the same ICA that we used [12]. This may be due to comparatively high parasite numbers and to the use of different PCR protocols. Interestingly, a poor sensitivity of another ICA (Meridian Bioscience, Inc., Cincinnati, OH, USA), ImmunoCard STAT, of 61% as compared with microscopy was obtained among Norwegian giardiasis patients with persistent symptoms following treatment [13]. Nonetheless, they also reported a considerable number of ICA-positive but microscopy-negative samples.

These discrepant results provide evidence that the value of ICAs in detecting *G. duodenalis* might differ considerably between populations and patient groups. The ICA evaluated by us may be helpful in settings comparable to Rwanda by identifying *G. duodenalis* infections with a higher sensitivity than microscopy; the true prevalence, however, is only realized after PCR testing. Notably, while the approximate direct costs (i.e. excluding laboratory staff and equipment) of light microscopy are low (0.2 €), those of ICA (3.5 €) and PCR (5–7 €) are substantial. Although the clinical relevance of detecting *G. duodenalis* at low numbers in largely asymptomatic children may be questionable on an individual level, the epidemiological relevance might be enormous, particularly regarding the transmission of the parasites.

Acknowledgements

We thank Steffen Zander for expert technical assistance, the children's families for participation in this study, and the staff at Sovu health centre, Kabutare district hospital, and CHUB central laboratory for their assistance and cooperation. The active assistance by the Rwanda office of the German Devel-

TABLE 1. Selected characteristics of 474 rural children with or without *Giardia duodenalis* infection detected by microscopy, immunochromatographic assay (ICA) or PCR

	Non-infected ($n = 153$)[a]	Microscopy positive ($n = 99$)	p	ICA positive ($n = 165$)	p	PCR positive ($n = 317$)	p	Total ($n = 474$)
% Febrile	3.9% (6/153)	4.1% (4/98)	1.0	4.3% (7/164)	0.88	3.2% (10/315)	0.68	3.4% (16/472)
% Loss of appetite (history)	11.1% (17/153)	12.1% (12/99)	0.81	10.9% (18/165)	0.95	11.7% (37/317)	0.86	11.4% (54/474)
% Diarrhoea (history)	6.5% (10/153)	3.0% (33/99)	0.22	7.3% (12/165)	0.80	6.6% (21/317)	0.97	6.5% (31/474)
% Vomiting (history)	2.0% (3/153)	1.0% (1/99)	0.56	1.2% (2/165)	0.59	2.2% (7/317)	0.86	2.1% (10/474)
% Abdominal pain (history)	3.3% (5/153)	5.1% (5/99)	0.48	5.5% (9/165)	0.34	4.7% (15/317)	0.46	4.2% (20/474)
% Abdominal distension (clinically)	1.3% (2/150)	5.1% (5/99)	0.12	5.5% (9/163)	0.04	6.4% (20/313)	0.02	4.7% (22/467)
% Severe malnutrition (clinically)	4.6% (7/151)	19.4% (19/98)	0.002	14.8% (24/162)	0.003	14.4% (45/313)	0.002	11.1% (52/468)
% Underweight[b]	19.7% (30/152)	29.6% (29/98)	0.07	25.0% (41/164)	0.26	24.7% (78/316)	0.23	23.1% (109/472)

[a]Children negative by microscopy, ICA and PCR ('non-infected') are compared with children positive by one of the three diagnostic methods.
[b]Weight-for-age z-score less than –2 SD.
Note: Columns add up to >474 because of overlap of samples found positive by the three diagnostic tests.

TABLE 2. The immunochromatographic assay (ICA) is more sensitive than light microscopy for the detection of *Giardia duodenalis* in Rwandan children (n = 558)

PCR	Microscopy n (%)	Positive	Sensitivity	Specificity	PPV	NPV	ICA Positive	Sensitivity	Specificity	PPV	NPV
Negative	199 (35.7)	2					7				
Positive	359 (64.3)	106	29.5 (24.9–34.6)*	99.0 (96.0–99.8)	98.1 (92.8–99.7)	43.8 (39.2–48.5)	181	50.4 (45.1–55.7)	96.5 (92.6–98.5)	96.3(92.2–98.4)	51.9 (46.7–57.1)

*Per cent plus 95% CI.
NPV, negative predictive value; PPV, positive predictive value.

opment Cooperation (GIZ) is gratefully acknowledged. This study was supported by the German Federal Ministry for Economic Cooperation and Development through the ESTHER programme (*Ensemble pour une Solidarité Thérapeutique Hospitalière En Réseau*) and by the German Federal Ministry of Education and Research (grant 01DG13006A, MOPACUR).

Transparency Declaration

The authors declare no conflicts of interest.

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